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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
7 November 2002 (07.11.2002)

PCT

(10) International Publication Number
WO 02/088307 A2

(51) International Patent Classification[7]: C12N

(21) International Application Number: PCT/US02/11854

(22) International Filing Date: 26 April 2002 (26.04.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/287,653 30 April 2001 (30.04.2001) US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT (utility model), AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ (utility model), CZ, DE (utility model), DE, DK (utility model), DK, DM, DZ, EC, EE (utility model), EE, ES, FI (utility model), FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK (utility model), SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/088307 A2

(54) Title: HUMANIZED ANTIBODIES

(57) Abstract: Humanized forms of mouse antibody 10D5 that retain the binding properties of mouse 10D5 are disclosed. Also disclosed are processes for making the humanized antibody, intermediates for making the humanized antibodies, including, nucleotide sequences, vectors, transformed host cells, and methods of using the humanized antibody to treat, prevent, alleviate, reverse, or otherwise ameliorate symptoms or pathology or both, that are associated with Down's syndrome or pre-clinical or clinical Alzheimer's disease or cerebral amyloid angiopathy.

HUMANIZED ANTIBODIES

This application claims priority of US 60/287,653, filed 2001 April 30, the entire contents of which are incorporated herein by reference.

The invention relates to humanized antibodies useful for treating and preventing human diseases associated with amyloid β (Aβ), such as Alzheimer's disease, Down's syndrome, and cerebral amyloid angiopathy. Mouse monoclonal antibody 10D5 was raised by immunizing mice with human Aβ1-28, and has been widely used in analytical methods [*J. Neuropathol. Exper. Neurology 51*:76-83 (1992); *Nature 359*:325-327 (1992); *Neuroscience Lett. 172*:122-124 (1994); *Biochem. Biophys. Res. Commun. 200*:1598-1603 (1994); *J. Neuropathol. Exper. Neurology 53*:377-383 (1994); *Annals Neurology 37*:512-518 (1995); *Annals Neurology 41*:809-813 (1997); *J. Neuroimmunol. 88*:85-90 (1998); *J. Neuroimmunol. 95*:136-142 (1999)]. 10D5 has been shown to bind to the N-terminal region of Aβ and has affinity of approximately 43 pM for aggregated Aβ.

After 10D5 was administered to a group of 8.5 to 10.5 month-old heterozygous, transgenic PDAPP mice (APP^{V717F}) at a weekly intraperitoneal dose of about 10 mg/kg for six months, the mice had significantly reduced levels of Aβ1-42 in brain cortex. However, the 10D5 group did not have a significant reduction of total Aβ in any tissue, nor of Aβ1-42 in hippocampus or cerebellum [Bard, F., *et al., Nature Med.* 6:916-919 (2000); WO 00/72876 and WO 00/72880, 7 December, 2000]. It was asserted that amyloid plaques in the 10D5 group also reduced in number and appearance, with some evidence of cell-associated immunoreactivity.

Another study in WO 00/72876 and WO 00/72880 reported that administration of 10D5 to older mice for six months caused a significant reduction in amyloid β plaque burden. It was asserted that the antibody gained access to the central nervous system in sufficient amounts to "decorate" β-amyloid plaques. Finally, it was stated that mouse 10D5 induces phagocytosis of amyloid plaques in *in vitro* studies.

Methods for administering aggregated Aβ1-42 to provoke an immunologic response and reduced amyloid deposits are described in PCT publication WO99/27944, published 10 June 1999. The description postulates that full-length aggregated Aβ peptide would be a useful immunogen. The application also indicates that antibodies that bind to Aβ peptide could be used as alternate therapeutic agents. However, this appears

to be speculation since the supporting data reflect protocols that involve active immunization using, for example, Aβ1–42.

WO 99/60024, published 25 November 1999, is directed to methods for amyloid removal using anti-amyloid antibodies. The mechanism, however, is stated to utilize the ability of anti-Aβ antibodies to bind to pre-formed amyloid deposits (i.e. plaques) and result in subsequent microglial clearance of localized plaques. This mechanism was not proved *in vivo*. This publication further states that to be effective against Aβ plaques, anti-Aβ antibodies must be delivered directly to the brain, because antibodies cannot cross the blood brain barrier.

Queen, *et al.* describe methods of humanizing antibodies [*e.g.*, US Patent Nos. 5,585,089, 5,693,761, 5,693,762, 6,180,370].

Humanized forms of 10D5 are needed for use in humans having Down's syndrome, or pre-clinical or clinical Alzheimer's disease or cerebral amyloid angiopathy (CAA). However, it is not known whether 10D5 can be humanized so that the humanized antibody retained the binding properties of the mouse antibody.

Summary of the Invention

This invention provides humanized forms of 10D5. These humanized antibodies have binding properties (affinity and epitope location) that are approximately the same as those of the mouse 10D5 antibody. The invention includes antibodies, single chain antibodies, and fragments thereof. The invention includes antibodies wherein the CDR are those of mouse monoclonal antibody 10D5 (sequences SEQ ID NO:1 through SEQ ID NO:6) and wherein the antibodies retain approximately the binding properties of the mouse antibody and have *in vitro* and *in vivo* properties functionally equivalent to the mouse antibody. In another aspect, this invention provides humanized antibodies and fragments thereof, wherein the variable regions have sequences comprising the CDR from mouse antibody 10D5 and specific human framework sequences (sequences SEQ ID NO:7 - SEQ ID NO:10), wherein the antibodies retain approximately the binding properties of the mouse antibody and have *in vitro* and *in vivo* properties functionally equivalent to the mouse antibody 10D5. In another aspect, this invention provides humanized antibodies and fragments thereof, wherein the light chain is SEQ ID NO:11 and the heavy chain is SEQ ID NO:12.

Also part of the invention are polynucleotide sequences that encode the humanized antibodies or fragments thereof disclosed above, vectors comprising the polynucleotide sequences encoding the humanized antibodies or fragments thereof, host cells transformed with the vectors or incorporating the polynucleotides that express the humanized antibodies or fragments thereof, pharmaceutical formulations of the humanized antibodies and fragments thereof disclosed herein, and methods of making and using the same.

Such humanized antibodies and fragments thereof are useful for, among other things, treating and preventing diseases and conditions characterized by Aβ plaques or Aβ toxicity in the brain, such as Alzheimer's disease, Down's syndrome, and cerebral amyloid angiopathy in humans.

The invention also includes use of a humanized antibody of the present invention for the manufacture of a medicament, including prolonged expression of recombinant sequences of the antibody or antibody fragment in human tissues, for treating, preventing, or reversing Alzheimer's disease, Down's syndrome, or cerebral amyloid angiopathy, or to inhibit the formation of amyloid plaques or the effects of toxic soluble Aβ species in humans.

Detailed Description of the Invention

We have surprisingly found that humanized antibodies, wherein the CDRs originate from mouse monoclonal antibody 10D5 and the framework and other portions of the antibodies originate from a human germ line, bind Aβ1-40 and Aβ1-42 with at least the affinity with which mouse 10D5 binds Aβ. Thus, we have a reasonable basis for believing that humanized antibodies of this specificity, modified to reduce their immunogenicity by converting them to a humanized form, offer the opportunity to treat, both prophylactically and therapeutically, conditions in humans that are associated with formation of beta-amyloid plaques. These conditions include, as noted above, pre-clinical and clinical Alzheimer's, Down's syndrome, and pre-clinical and clinical cerebral amyloid angiopathy.

As used herein, the word "treat" includes therapeutic treatment, where a condition to be treated is already known to be present and prophylaxis - *i.e.*, prevention of, or amelioration of, the possible future onset of a condition.

By "antibody" is meant a monoclonal antibody *per se*, or an immunologically effective fragment thereof, such as an Fab, Fab', or $F(ab')_2$ fragment thereof. In some contexts, herein, fragments will be mentioned specifically for emphasis; nevertheless, it will be understood that regardless of whether fragments are specified, the term "antibody" includes such fragments as well as single-chain forms. As long as the protein retains the ability specifically to bind its intended target, it is included within the term "antibody." Also included within the definition "antibody" are single chain forms. Preferably, but not necessarily, the antibodies useful in the invention are produced recombinantly. Antibodies may or may not be glycosylated, though glycosylated antibodies are preferred. Antibodies are properly cross-linked via disulfide bonds, as is well known.

The basic antibody structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function.

Light chains are classified as kappa and lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, and define the antibody's isotype as IgG, IgM, IgA, IgD and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 3 or more amino acids.

The variable regions of each light/heavy chain pair form the antibody binding site. Thus, an intact antibody has two binding sites. The chains all exhibit the same general structure of relatively conserved framework regions (FR) joined by three hypervariable regions, also called complementarity determining regions or CDRs. The CDRs from the two chains of each pair are aligned by the framework regions, enabling binding to a specific epitope. From N- terminal to C-terminal, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with well known conventions [Kabat, et al., "Sequences of Proteins of Immunological Interest" National Institutes of Health, Bethesda, Md., 1987 and 1991; Chothia, et al., J. Mol. Biol. 196:901-917 (1987); Chothia, et al., Nature 342:878-883 (1989)].

By "humanized antibody" is meant an antibody that is composed partially or fully of amino acid sequences derived from a human antibody germline by altering the sequence of an antibody having non-human complementarity determining regions (CDR). A humanized immunoglobulin does not encompass a chimeric antibody, having a mouse variable region and a human constant region. However, the variable region of the antibody and even the CDR are humanized by techniques that are by now well known in the art. The framework regions of the variable regions are substituted by the corresponding human framework regions leaving the non-human CDR substantially intact. As mentioned above, it is sufficient for use in the methods of the invention, to employ an immunologically specific fragment of the antibody, including fragments representing single chain forms.

Humanized antibodies have at least three potential advantages over non-human and chimeric antibodies for use in human therapy:

1) because the effector portion is human, it may interact better with the other parts of the human immune system (e.g., destroy the target cells more efficiently by complement-dependent cytotoxicity (CDC) or antibody-dependent cellular cytotoxicity (ADCC).

2) The human immune system should not recognize the framework or C region of the humanized antibody as foreign, and therefore the antibody response against such an injected antibody should be less than against a totally foreign non-human antibody or a partially foreign chimeric antibody.

3) Injected non-human antibodies have been reported to have a half-life in the human circulation much shorter than the half-life of human antibodies. Injected humanized antibodies will have a half-life essentially identical to naturally occurring human antibodies, allowing smaller and less frequent doses to be given.

The design of humanized immunoglobulins may be carried out as follows. As to the human framework region, a framework or variable region amino acid sequence of a CDR-providing non-human immunoglobulin is compared with corresponding sequences in a human immunoglobulin variable region sequence collection, and a sequence having a high percentage of identical amino acids is selected. When an amino acid falls under the following category, the framework amino acid of a human immunoglobulin to be used

(acceptor immunoglobulin) is replaced by a framework amino acid from a CDR-providing non-human immunoglobulin (donor immunoglobulin):

(a) the amino acid in the human framework region of the acceptor immunoglobulin is unusual for human immunoglobulin at that position, whereas the corresponding amino acid in the donor immunoglobulin is typical for human immunoglobulin at that position;

(b) the position of the amino acid is immediately adjacent to one of the CDRs; or

(c) any side chain atom of a framework amino acid is within about 5-6 angstroms (center-to-center) of any atom of a CDR amino acid in a three dimensional immunoglobulin model [Queen, *et al.*, Proc. Natl. Acad. Sci. USA 86:10029-10033 (1989), and Co, et al., Proc. Natl. Acad. Sci. USA 88, 2869 (1991)]. When each of the amino acid in the human framework region of the acceptor immunoglobulin and a corresponding amino acid in the donor immunoglobulin is unusual for human immunoglobulin at that position, such an amino acid is replaced by an amino acid typical for human immunoglobulin at that position.

A preferred humanized antibody is a humanized form of mouse antibody 10D5. The CDRs of humanized 10D5 have the following amino acid sequences:

light chain CDR1:

```
 1               5                  10                  15
Arg Ser Ser Gln Asn Ile Ile His Ser Asn Gly Asn Thr Tyr Leu Glu
(SEQ ID NO:1)
```

light chain CDR2:

```
 1               5
Lys Val Ser Asn Arg Phe Ser  (SEQ ID NO:2)
```

light chain CDR3:

```
1               5
Phe Gln Gly Ser His Val Pro Leu Thr  (SEQ ID NO:3)
```

heavy chain CDR1:

```
 1               5
Thr Ser Gly Met Gly Val Ser (SEQ ID NO:4)
```

heavy chain CDR2:

```
 1               5                  10                  15
His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ser Leu Lys Ser
(SEQ ID NO:5)
```

and, heavy chain CDR3:

```
 1               5                  10
Arg Pro Ile Thr Pro Val Leu Val Asp Ala Met Asp Tyr  (SEQ ID NO:6).
```

A preferred light chain variable region of a humanized antibody of the present invention has the following amino acid sequence, in which the framework originated from human germline Vk segment DPK18 and J segment Jk4:

Asp Val Xaa Met Thr Gln Xaa Pro Leu Ser Leu Pro Val Xaa Leu Gly
Xaa Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile Xaa His Ser
Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
Pro Xaa Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
Ser Arg Val Glu Ala Glu Asp Xaa Gly Val Tyr Tyr Cys Phe Gln Gly
Ser His Val Pro Leu Thr Phe Gly Xaa Gly Thr Lys Xaa Glu Ile Lys
Arg (SEQ ID NO:7).

wherein:

Xaa at position 3 is Val or Leu;

Xaa at position 7 is Ser or Thr;

Xaa at position 14 is Thr or Ser;

Xaa at position 17 is Gln, Asp, or Asn;

Xaa at position 30 is Ile or Val;

Xaa at position 50 is Arg or Lys;

Xaa at position 88 is Val or Leu;

Xaa at position 105 is Gly or Ala; and

Xaa at position 109 is Val or Leu.

A preferred heavy chain variable region of a humanized antibody of the present invention has the following amino acid sequence, in which the framework originated from human germline VH segment DP-28 and J segment JH4, with several amino acid substitutions to the consensus amino acids in the same human subgroup to reduce potential immunogenicity:

```
1               5                  10                  15
Xaa Xaa Thr Leu Lys Glu Ser Gly Pro Val Leu Val Lys Pro Thr Glu

            20                  25                  30
Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ser

        35                  40                  45
Gly Met Gly Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu

    50                  55                  60
Trp Leu Ala His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Xaa

65                  70                  75                  80
Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Xaa Xaa Gln Val

                85                  90                  95
Val Leu Xaa Xaa Thr Xaa Xaa Asp Pro Val Asp Thr Ala Thr Tyr Tyr

            100                 105                 110
Cys Val Arg Arg Pro Ile Thr Pro Val Leu Val Asp Ala Met Asp Tyr

        115                 120
Trp Gly Gln Gly Thr Xaa Val Thr Val Ser Ser            (SEQ ID NO:8).
```

wherein:

Xaa at position 1 is Gln or Glu;

Xaa at position 2 is Val or Ala;

Xaa at position 64 is Ser or Thr;

Xaa at position 77 is Lys or Arg;

Xaa at position 78 is Ser or Thr;

Xaa at position 83 is Thr or Ser;

Xaa at position 84 is Met, Ile, or Leu;

Xaa at position 86 is Asn, Ser, or Thr;

Xaa at position 87 is Met, Val, or Leu; and

Xaa at position 118 is Leu or Ser.

A particularly preferred light chain variable region of a humanized antibody of the present invention has the following amino acid sequence, in which the framework originated from human germline Vk segment DPK18 and J segment Jk4:

```
1               5                  10                  15
Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly

            20                  25                  30
Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile Ile His Ser

        35                  40                  45
Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
```

```
     50                  55                  60
Pro Arg Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro

 65                  70                  75                  80
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile

                85                  90                  95
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly

            100                 105                 110
Ser His Val Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys

Arg                                                    (SEQ ID NO:9).
```

A particularly preferred heavy chain variable region of a humanized antibody of the present invention has the following amino acid sequence, in which the framework originated from human germline VH segment DP-28 and J segment JH4:

```
 1               5                  10                  15
Gln Val Thr Leu Lys Glu Ser Gly Pro Val Leu Val Lys Pro Thr Glu

            20                  25                  30
Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ser

        35                  40                  45
Gly Met Gly Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu

    50                  55                  60
Trp Leu Ala His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ser

 65                  70                  75                  80
Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Ser Gln Val

                85                  90                  95
Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr

            100                 105                 110
Cys Val Arg Arg Pro Ile Thr Pro Val Leu Val Asp Ala Met Asp Tyr

        115                 120
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser             (SEQ ID NO:10).
```

A preferred light chain for a humanized antibody of the present invention has the amino acid sequence:

```
 1               5                  10                  15
Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly

            20                  25                  30
Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile Ile His Ser
```

```
          35                  40                  45
Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser

      50                  55                  60
Pro Arg Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro

 65                  70                  75                  80
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile

                 85                  90                  95
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly

            100                 105                 110
Ser His Val Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys

        115                 120                 125
Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu

    130                 135                 140
Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe

145                 150                 155                 160
Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln

                165                 170                 175
Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser

            180                 185                 190
Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu

        195                 200                 205
Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser

    210                 215
Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys            (SEQ ID NO:11).
```

A preferred heavy chain for a humanized antibody of the present invention has the amino acid sequence:

```
 1               5                  10                  15
Gln Val Thr Leu Lys Glu Ser Gly Pro Val Leu Val Lys Pro Thr Glu

            20                  25                  30
Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ser

        35                  40                  45
Gly Met Gly Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu

    50                  55                  60
Trp Leu Ala His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ser

 65                  70                  75                  80
Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Ser Gln Val

                 85                  90                  95
Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr

            100                 105                 110
Cys Val Arg Arg Pro Ile Thr Pro Val Leu Val Asp Ala Met Asp Tyr
```

```
          115                 120                 125
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly

      130                 135                 140
Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly

145                 150                 155                 160
Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val

                165                 170                 175
Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe

            180                 185                 190
Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val

        195                 200                 205
Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val

    210                 215                 220
Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys

225                 230                 235                 240
Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu

                245                 250                 255
Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr

            260                 265                 270
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val

        275                 280                 285
Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val

    290                 295                 300
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser

305                 310                 315                 320
Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu

                325                 330                 335
Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala

            340                 345                 350
Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro

        355                 360                 365
Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln

    370                 375                 380
Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala

385                 390                 395                 400
Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr

                405                 410                 415
Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu

            420                 425                 430
Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser

        435                 440                 445
Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
```

450
Leu Ser Pro Gly Lys (SEQ ID NO:12).

Other sequences are possible for the light and heavy chains for humanized 10D5. The immunoglobulins can have two pairs of light chain/heavy chain complexes, at least one chain comprising one or more mouse complementarity determining regions functionally joined to human framework region segments.

In another aspect, the present invention is directed to recombinant polynucleotides encoding antibodies which, when expressed, comprise the heavy and light chain CDRs from an antibody of the present invention. Exemplary polynucleotides, which on expression code for the polypeptide chains comprising the heavy and light chain CDRs of monoclonal antibody 10D5 are given herein. Due to codon degeneracy, other polynucleotide sequences can be readily substituted for those sequences. Particularly preferred polynucleotides of the present invention encode antibodies, which when expressed, comprise the CDRs of SEQ ID NO:1 – SEQ ID NO:6, or any of the variable regions of SEQ ID NO:7 – SEQ ID NO:10, or the light and heavy chains of SEQ ID NO:11 and SEQ ID NO:12.

The polynucleotides will typically further include an expression control polynucleotide sequence operably linked to the humanized immunoglobulin coding sequences, including naturally-associated or heterologous promoter regions. Preferably, the expression control sequences will be eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells, but control sequences for prokaryotic hosts may also be used. Once the vector has been incorporated into the appropriate host cell line, the host cell is propagated under conditions suitable for expressing the nucleotide sequences, and, as desired, the collection and purification of the light chains, heavy chains, light/heavy chain dimers or intact antibodies, binding fragments or other immunoglobulin forms may follow.

The nucleic acid sequences of the present invention capable of ultimately expressing the desired humanized antibodies can be formed from a variety of different polynucleotides (genomic or cDNA, RNA, synthetic oligonucleotides, etc.) and components (e.g., V, J, D, and C regions), using any of a variety of well known

techniques. Joining appropriate genomic and synthetic sequences is a common method of production, but cDNA sequences may also be utilized.

Below is a cDNA sequence (SEQ ID NO:17), from which the light chain having the amino acid sequence of SEQ ID NO:19 may be expressed.

```
          ATGAAGTTGCCTGTTAGGCTGTTGGTACTGATGTTCTGGATTCCTGCTTCCAGCAGTGAT
        1 ---------+---------+---------+---------+---------+---------+ 60
          M  K  L  P  V  R  L  L  V  L  M  F  W  I  P  A  S  S  S  D  -

          GTTGTGATGACCCAATCTCCACTCTCCCTGCCTGTCACTCTTGGACAGCCAGCCTCCATC
       61 ---------+---------+---------+---------+---------+---------+ 120
          V  V  M  T  Q  S  P  L  S  L  P  V  T  L  G  Q  P  A  S  I  -

          TCTTGCAGATCTAGTCAGAACATTATACATAGTAATGGAAACACCTATTTAGAATGGTAC
      121 ---------+---------+---------+---------+---------+---------+ 180
          S  C  R  S  S  Q  N  I  I  H  S  N  G  N  T  Y  L  E  W  Y  -

          CTGCAGAAACCAGGCCAGTCTCCAAGGCTCCTGATCTACAAAGTTTCCAACCGATTTTCT
      181 ---------+---------+---------+---------+---------+---------+ 240
          L  Q  K  P  G  Q  S  P  R  L  L  I  Y  K  V  S  N  R  F  S  -

          GGGGTCCCAGACAGGTTCAGTGGCAGTGGATCAGGGACAGATTTCACACTCAAGATCAGC
      241 ---------+---------+---------+---------+---------+---------+ 300
          G  V  P  D  R  F  S  G  S  G  S  G  T  D  F  T  L  K  I  S  -

          AGAGTGGAGGCTGAGGATGTGGGAGTTTATTACTGCTTTCAAGGTTCACATGTTCCGCTC
      301 ---------+---------+---------+---------+---------+---------+ 360
          R  V  E  A  E  D  V  G  V  Y  Y  C  F  Q  G  S  H  V  P  L  -

          ACTTTCGGCGGAGGGACCAAGGTGGAAATAAAACGAACTGTGGCTGCACCATCTGTCTTC
      361 ---------+---------+---------+---------+---------+---------+ 420
          T  F  G  G  G  T  K  V  E  I  K  R  T  V  A  A  P  S  V  F  -

          ATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTG
      421 ---------+---------+---------+---------+---------+---------+ 480
          I  F  P  P  S  D  E  Q  L  K  S  G  T  A  S  V  V  C  L  L  -

          AATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCG
      481 ---------+---------+---------+---------+---------+---------+ 540
          N  N  F  Y  P  R  E  A  K  V  Q  W  K  V  D  N  A  L  Q  S  -

          GGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGC
      541 ---------+---------+---------+---------+---------+---------+ 600
          G  N  S  Q  E  S  V  T  E  Q  D  S  K  D  S  T  Y  S  L  S  -

          AGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTC
      601 ---------+---------+---------+---------+---------+---------+ 660
          S  T  L  T  L  S  K  A  D  Y  E  K  H  K  V  Y  A  C  E  V  -

          ACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT    (SEQ ID NO:17)
      661 ---------+---------+---------+---------+---------+----  714
          T  H  Q  G  L  S  S  P  V  T  K  S  F  N  R  G  E  C   - (SEQ ID NO:19)
```

Below is a cDNA sequence (SEQ ID NO:18), from which the heavy chain having the amino acid sequence of SEQ ID NO:20 may be expressed.

```
          ATGGACAGGCTTACTTCCTCATTCCTGCTGCTGATTGTCCCTGCATATGTCCTGTCCCAG
        1 ---------+---------+---------+---------+---------+---------+ 60
          M  D  R  L  T  S  S  F  L  L  L  I  V  P  A  Y  V  L  S  Q  -
```

```
    GTTACTCTGAAAGAGTCTGGCCCTGTACTAGTGAAGCCCACCGAGACCCTCACTCTGACT
 61 ---------+---------+---------+---------+---------+---------+ 120
    V  T  L  K  E  S  G  P  V  L  V  K  P  T  E  T  L  T  L  T  -

    TGTACTTTCTCTGGGTTTTCACTGAGCACTTCTGGTATGGGAGTGAGCTGGATTCGTCAG
121 ---------+---------+---------+---------+---------+---------+ 180
    C  T  F  S  G  F  S  L  S  T  S  G  M  G  V  S  W  I  R  Q  -

    CCTCCAGGAAAGGCTCTGGAGTGGCTGGCACACATTTACTGGGATGATGACAAGCGCTAT
181 ---------+---------+---------+---------+---------+---------+ 240
    P  P  G  K  A  L  E  W  L  A  H  I  Y  W  D  D  D  K  R  Y  -

    AACCCATCCCTGAAGAGCCGGCTCACAATCTCCAAGGATACCTCCAAAAGCCAGGTAGTC
241 ---------+---------+---------+---------+---------+---------+ 300
    N  P  S  L  K  S  R  L  T  I  S  K  D  T  S  K  S  Q  V  V  -

    CTCACGATGACCAATATGGACCCTGTAGATACTGCCACATACTACTGTGTTCGAAGGCCC
301 ---------+---------+---------+---------+---------+---------+ 360
    L  T  M  T  N  M  D  P  V  D  T  A  T  Y  Y  C  V  R  R  P  -

    ATTACTCCGGTACTAGTCGATGCTATGGACTACTGGGGCCAAGGAACCCTGGTCACCGTC
361 ---------+---------+---------+---------+---------+---------+ 420
    I  T  P  V  L  V  D  A  M  D  Y  W  G  Q  G  T  L  V  T  V  -

    TCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACC
421 ---------+---------+---------+---------+---------+---------+ 480
    S  S  A  S  T  K  G  P  S  V  F  P  L  A  P  S  S  K  S  T  -

    TCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACG
481 ---------+---------+---------+---------+---------+---------+ 540
    S  G  G  T  A  A  L  G  C  L  V  K  D  Y  F  P  E  P  V  T  -

    GTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAG
541 ---------+---------+---------+---------+---------+---------+ 600
    V  S  W  N  S  G  A  L  T  S  G  V  H  T  F  P  A  V  L  Q  -

    TCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACC
601 ---------+---------+---------+---------+---------+---------+ 660
    S  S  G  L  Y  S  L  S  S  V  V  T  V  P  S  S  S  L  G  T  -

    CAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTT
661 ---------+---------+---------+---------+---------+---------+ 720
    Q  T  Y  I  C  N  V  N  H  K  P  S  N  T  K  V  D  K  K  V  -

    GAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTG
721 ---------+---------+---------+---------+---------+---------+ 780
    E  P  K  S  C  D  K  T  H  T  C  P  P  C  P  A  P  E  L  L  -

    GGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGG
781 ---------+---------+---------+---------+---------+---------+ 840
    G  G  P  S  V  F  L  F  P  P  K  P  K  D  T  L  M  I  S  R  -

    ACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTC
841 ---------+---------+---------+---------+---------+---------+ 900
    T  P  E  V  T  C  V  V  V  D  V  S  H  E  D  P  E  V  K  F  -

    AACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAG
901 ---------+---------+---------+---------+---------+---------+ 960
    N  W  Y  V  D  G  V  E  V  H  N  A  K  T  K  P  R  E  E  Q  -

    TACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAAT
961 ---------+---------+---------+---------+---------+---------+ 1020
    Y  N  S  T  Y  R  V  V  S  V  L  T  V  L  H  Q  D  W  L  N  -

    GGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACC
1021 ---------+---------+---------+---------+---------+---------+ 1080
    G  K  E  Y  K  C  K  V  S  N  K  A  L  P  A  P  I  E  K  T  -
```

```
     ATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGG
1081 ---------+---------+---------+---------+---------+---------+ 1140
     I  S  K  A  K  G  Q  P  R  E  P  Q  V  Y  T  L  P  P  S  R   -

     GATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGC
1141 ---------+---------+---------+---------+---------+---------+ 1200
     D  E  L  T  K  N  Q  V  S  L  T  C  L  V  K  G  F  Y  P  S   -

     GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCT
1201 ---------+---------+---------+---------+---------+---------+ 1260
     D  I  A  V  E  W  E  S  N  G  Q  P  E  N  N  Y  K  T  T  P   -

     CCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGC
1261 ---------+---------+---------+---------+---------+---------+ 1320
     P  V  L  D  S  D  G  S  F  F  L  Y  S  K  L  T  V  D  K  S   -

     AGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCAC
1321 ---------+---------+---------+---------+---------+---------+ 1380
     R  W  Q  Q  G  N  V  F  S  C  S  V  M  H  E  A  L  H  N  H   -

     TACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA          (SEQ ID NO:18)
1381 ---------+---------+---------+------ 1416
     Y  T  Q  K  S  L  S  L  S  P  G  K   -        (SEQ ID NO:20)
```

The complete sequence of a humanized 10D5 light chain gene with introns (located between MluI and BamHI sites, as in pVk-Hu10D5) is shown below (SEQ ID NO:15). The nucleotide number indicates its position in pVk-Hu10D5. The V_k and C_k exons are translated in single letter code; the dot indicates the translation termination codon. The mature light chain starts at the double-underlined aspartic acid (D). The intron sequences are in italic. The expressed light chain corresponds to SEQ ID NO:11 when mature.

```
 619 ACGCGTCCACCATGAAGTTGCCTGTTAGGCTGTTGGTACTGATGTTCTGGATTCCTGCTTCCAGCAGTGATGTTGTGATG
                   M  K  L  P  V  R  L  L  V  L  M  F  W  I  P  A  S  S  S  D  V  V  M
 699 ACCCAATCTCCACTCTCCCTGCCTGTCACTCTTGGACAGCCAGCCTCCATCTCTTGCAGATCTAGTCAGAACATTATACA
      T  Q  S  P  L  S  L  P  V  T  L  G  Q  P  A  S  I  S  C  R  S  S  Q  N  I  I  H
 779 TAGTAATGGAAACACCTATTTAGAATGGTACCTGCAGAAACCAGGCCAGTCTCCAAGGCTCCTGATCTACAAAGTTTCCA
       S  N  G  N  T  Y  L  E  W  Y  L  Q  K  P  G  Q  S  P  R  L  L  I  Y  K  V  S
 859 ACCGATTTTCTGGGGTCCCAGACAGGTTCAGTGGCAGTGGATCAGGGACAGATTTCACACTCAAGATCAGCAGAGTGGAG
      N  R  F  S  G  V  P  D  R  F  S  G  S  G  S  G  T  D  F  T  L  K  I  S  R  V  E
 939 GCTGAGGATGTGGGAGTTTATTACTGCTTTCAAGGTTCACATGTTCCGCTCACTTTCGGCGGAGGGACCAAGGTGGAAAT
       A  E  D  V  G  V  Y  Y  C  F  Q  G  S  H  V  P  L  T  F  G  G  G  T  K  V  E  I
1019 AAAACGTAAGTGCACTTTCCTAATCTAGAAATTCTAAACTCTGAGGGGGTCGGATGACGTGGCCATTCTTTGCCTAAAGC
       K  R
1099 ATTGAGTTTACTGCAAGGTCAGAAAAGCATGCAAAGCCCTCAGAATGGCTGCAAAGAGCTCCAACAAAACAATTTAGAAC
1179 TTTATTAAGGAATAGGGGGAAGCTAGGAAGAAACTCAAAACATCAAGATTTTAAATACGCTTCTTGGTCTCCTTGCTATA
1259 ATTATCTGGGATAAGCATGCTGTTTTCTGTCTGTCCCTAACATGCCCTGTGATTATCCGCAAACAACACACCCAAGGGCA
1339 GAACTTTGTTACTTAAACACCATCCTGTTTGCTTCTTTCCTCAGGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGC
                                                    T  V  A  A  P  S  V  F  I  F  P
1419 CATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTA
      P  S  D  E  Q  L  K  S  G  T  A  S  V  V  C  L  L  N  N  F  Y  P  R  E  A  K  V
1499 CAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTA
       Q  W  K  V  D  N  A  L  Q  S  G  N  S  Q  E  S  V  T  E  Q  D  S  K  D  S  T  Y
1579 CAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGG
       S  L  S  S  T  L  T  L  S  K  A  D  Y  E  K  H  K  V  Y  A  C  E  V  T  H  Q
1659 GCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAGAGGGAGAAGTGCCCCCACCTGCTCCTCAGTTCC
      G  L  S  S  P  V  T  K  S  F  N  R  G  E  C  •
1739 AGCCTGACCCCCTCCCATCCTTTGGCCTCTGACCCTTTTTCCACAGGGGACCTACCCCTATTGCGGTCCTCCAGCTCATC
1819 TTTCACCTCACCCCCCTCCTCCTCCTTGGCTTTAATTATGCTAATGTTGGAGGAGAATGAATAAATAAA GTGAATCTTTG
```

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1899 CACCTGTGGTTTCTCTCTTTCCTCATTTAATAATTATTATCTGTTGTTTTACCAACTACTCAATTTCTCTTATAAGGGAC
1979 TAAATATGTAGTCATCCTAAGGCGCATAACCATTTATAAAAATCATCCTTCATTCTATTTTACCCTATCATCCTCTGCAA
2059 GACAGTCCTCCCTCAAACCCACAAGCCTTCTGTCCTCACAGTCCCCTGGGCCATGGTAGGAGAGACTTGCTTCCTTGTTT
2139 TCCCCTCCTCAGCAAGCCCTCATAGTCCTTTTTAAGGGTGACAGGTCTTACAGTCATATATCCTTTGATTCAATTCCCTG
2219 AGAATCAACCAAAGCAAATTTTTCAAAAGAAGAAACCTGCTATAAAGAGAATCATTCATTGCAACATGATATAAAATAAC
2299 AACACAATAAAAGCAATTAAATAAACAAACAATAGGGAAATGTTTAAGTTCATCATGGTACTTAGACTTAATGGAATGTC
2379 ATGCCTTATTTACATTTTTAAACAGGTACTGAGGGACTCCTGTCTGCCAAGGGCCGTATTGAGTACTTTCCACAACCTAA
2459 TTTAATCCACACTATACTGTGAGATTAAAAACATTCATTAAAATGTTGCAAAGGTTCTATAAAGCTGAGAGACAAATATA
2539 TTCTATAACTCAGCAATCCCACTTCTAGGATCC                    (SEQ ID NO:15)
```

The complete sequence of a humanized 10D5 heavy chain gene with introns (located between MluI and BamHI sites, as in pVg1-Hu10D5) is shown below (SEQ ID NO:16). The nucleotide number indicates its position in pVg1-Hu10D5. The V_H and C_H exons are translated in single letter code; the dot indicates the translation termination codon. The mature heavy chain starts at the double-underlined glutamine (Q). The intron sequences are in italic. The expressed heavy chain corresponds to SEQ ID NO:12 when mature.

```
 619 ACGCGTCCACCATGGACAGGCTTACTTCCTCATTCCTGCTGCTGATTGTCCCTGCATATGTCCTGTCCCAGGTTACTCTG
                M  D  R  L  T  S  S  F  L  L  L  I  V  P  A  Y  V  L  S  Q  .  V  T  L
 699 AAAGAGTCTGGCCCTGTACTAGTGAAGCCCACCGAGACCCTCACTCTGACTTGTACTTTCTCTGGGTTTTCACTGAGCAC
     K  E  S  G  P  V  L  V  K  P  T  E  T  L  T  L  T  C  T  F  S  G  F  S  L  S  T
 779 TTCTGGTATGGGAGTGAGCTGGATTCGTCAGCCTCCAGGAAAGGCTCTGGAGTGGCTGGCACACATTTACTGGGATGATG
       S  G  M  G  V  S  W  I  R  Q  P  P  G  K  A  L  E  W  L  A  H  I  Y  W  D  D
 859 ACAAGCGCTATAACCCATCCCTGAAGAGCCGGCTCACAATCTCCAAGGATACCTCCAAAAGCCAGGTAGTCCTCACGATG
     D  K  R  Y  N  P  S  L  K  S  R  L  T  I  S  K  D  T  S  K  S  Q  V  V  L  T  M
 939 ACCAATATGGACCCTGTAGATACTGCCACATACTACTGTGTTCGAAGGCCCATTACTCCGGTACTAGTCGATGCTATGGA
       T  N  M  D  P  V  D  T  A  T  Y  Y  C  V  R  R  P  I  T  P  V  L  V  D  A  M  D
1019 CTACTGGGGCCAAGGAACCCTGGTCACCGTCTCCTCAGGTGAGTCCTCACAACCTCTAGAGCTTTCTGGGGCAGGCCAGG
       Y  W  G  Q  G  T  L  V  T  V  S  S
1099 CCTGACCTTGGCTTTGGGGCAGGGAGGGGGCTAAGGTGAGGCAGGTGGCGCCAGCCAGGTGCACACCCAATGCCCATGAG
1179 CCCAGACACTGGACGCTGAACCTCGCGGACAGTTAAGAACCCAGGGGCCTCTGCGCCCTGGGCCCAGCTCTGTCCCACAC
1259 CGCGGTCACATGGCACCACCTCTCTTGCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGC
                                  A  S  T  K  G  P  S  V  F  P  L  A  P  S  S  K  S
1339 ACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGG
       T  S  G  G  T  A  A  L  G  C  L  V  K  D  Y  F  P  E  P  V  T  V  S  W  N  S  G
1419 CGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCG
         A  L  T  S  G  V  H  T  F  P  A  V  L  Q  S  S  G  L  Y  S  L  S  S  V  V  T
1499 TGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAA
     V  P  S  S  S  L  G  T  Q  T  Y  I  C  N  V  N  H  K  P  S  N  T  K  V  D  K  K
1579 GTTGGTGAGAGGCCAGCACAGGGAGGGAGGGTGTCTGCTGGAAGCCAGGCTCAGCGCTCCTGCCTGGACGCATCCCGGCT
       V
1659 ATGCAGCCCCAGTCCAGGGCAGCAAGGCAGGCCCCGTCTGCCTCTTCACCCGGAGGCCTCTGCCCGCCCCACTCATGCTC
1739 AGGGAGAGGGTCTTCTGGCTTTTTCCCCAGGCTCTGGGCAGGCACAGGCTAGGTGCCCCTAACCCAGGCCCTGCACACAA
1819 AGGGGCAGGTGCTGGGCTCAGACCTGCCAAGAGCCATATCCGGGAGGACCCTGCCCCTGACCTAAGCCCACCCCAAAGGC
1899 CAAACTCTCCACTCCCTCAGCTCGGACACCTTCTCTCCTCCCAGATTCCAGTAACTCCCAATCTTCTCTCTGCAGAGCCC
                                                                                E  P
1979 AAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGGTAAGCCAGCCCAGGCCTCGCCCTCCAGCTCAAGGCGGGA
       K  S  C  D  K  T  H  T  C  P  P  C  P
2059 CAGGTGCCCTAGAGTAGCCTGCATCCAGGGACAGGCCCCAGCCGGGTGCTGACACGTCCACCTCCATCTCTTCCTCAGCA
                                                                                  A
2139 CCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGA
       P  E  L  L  G  G  P  S  V  F  L  F  P  P  K  P  K  D  T  L  M  I  S  R  T  P  E
2219 GGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGC
       V  T  C  V  V  V  D  V  S  H  E  D  P  E  V  K  F  N  W  Y  V  D  G  V  E  V
2299 ATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAG
     H  N  A  K  T  K  P  R  E  E  Q  Y  N  S  T  Y  R  V  V  S  V  L  T  V  L  H  Q
2379 GACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAA
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      D  W  L  N  G  K  E  Y  K  C  K  V  S  N  K  A  L  P  A  P  I  E  K  T  I  S  K
2459  AGCCAAAGGTGGGACCCGTGGGGTGCGAGGGCCACATGGACAGAGGCCGGCTCGGCCCACCCTCTGCCCTGAGAGTGACC
        A  K
2539  GCTGTACCAACCTCTGTCCCTACAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGAC
                                G  Q  P  R  E  P  Q  V  Y  T  L  P  P  S  R  D  E  L  T
2619  CAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGC
         K  N  Q  V  S  L  T  C  L  V  K  G  F  Y  P  S  D  I  A  V  E  W  E  S  N  G
2699  AGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTG
         Q  P  E  N  N  Y  K  T  T  P  P  V  L  D  S  D  G  S  F  F  L  Y  S  K  L  T  V
2779  GACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAA
         D  K  S  R  W  Q  Q  G  N  V  F  S  C  S  V  M  H  E  A  L  H  N  H  Y  T  Q  K
2859  GAGCCTCTCCCTGTCTCCGGGTAAATGAGTGCGACGGCCGGCAAGCCCCCGCTCCCCGGGCTCTCGCGGTCGCACGAGGA
         S  L  S  L  S  P  G  K  •
2939  TGCTTGGCACGTACCCCCTGTACATACTTCCCGGGCGCCCAGCATGGAAATAAA_GCACCCAGCGCTGCCCTGGGCCCCTG
3019  CGAGACTGTGATGGTTCTTTCCACGGGTCAGGCCGAGTCTGAGGCCTGAGTGGCATGAGGGAGGCAGAGCGGGTCCCACT
3099  GTCCCCACACTGGCCCAGGCTGTGCAGGTGTGCCTGGGCCGCCTAGGGTGGGGCTCAGCCAGGGGCTGCCCTCGGCAGGG
3179  TGGGGGATTTGCCAGCGTGGCCCTCCCTCCAGCAGCACCTGCCCTGGGCTGGGCCACGGGAAGCCCTAGGAGCCCCTGGG
3259  GACAGACACACAGCCCCTGCCTCTGTAGGAGACTGTCCTGTTCTGTGAGCGCCCTGTCCTCCGACCTCCATGCCCACTCG
3339  GGGGCATGCCTAGTCCATGTGCGTAGGGACAGGCCCTCCCTCACCCATCTACCCCCACGGCACTAACCCCTGGCTGCCCT
3419  GCCCAGCCTCGCACCCGCATGGGGACACAACCGACTCCGGGGACATGCACTCTCGGGCCCTGTGGAGGGACTGGTGCAGA
3499  TGCCCACACACACACTCAGCCCAGACCCGTTCAACAAACCCCGCACTGAGGTTGGCCGGCCACACGGCCACCACACACAC
3579  ACGTGCACGCCTCACACACGGAGCCTCACCCGGGCGAACTGCACAGCACCCAGACCAGAGCAAGGTCCTCGCACACGTGA
3659  ACACTCCTCGGACACAGGCCCCCACGAGCCCCACGCGGCACCTCAAGGCCCACGAGCCTCTCGGCAGCTTCTCCACATGC
3739  TGACCTGCTCAGACAAACCCAGCCCTCCTCTCACAAGGGTGCCCCTGCAGCCGCCACACACACACAGGGGATCACACACC
3819  ACGTCACGTCCCTGGCCCTGGCCCACTTCCCAGTGCCGCCCTTCCCTGCAGGATCC          (SEQ ID NO:16)
```

Human constant region DNA sequences can be isolated in accordance with well known procedures from a variety of human cells, but preferably from immortalized B-cells. Suitable source cells for the polynucleotide sequences and host cells for immunoglobulin expression and secretion can be obtained from a number of sources well-known in the art.

In addition to the humanized immunoglobulins specifically described herein, other "substantially homologous" modified immunoglobulins can be readily designed and manufactured utilizing various recombinant DNA techniques well known to those skilled in the art. For example, the framework regions can vary from the native sequences at the primary structure level by several amino acid substitutions, terminal and intermediate additions and deletions, and the like. Moreover, a variety of different human framework regions may be used singly or in combination as a basis for the humanized immunoglobulins of the present invention. In general, modifications of the genes may be readily accomplished by a variety of well-known techniques, such as site-directed mutagenesis.

Alternatively, polypeptide fragments comprising only a portion of the primary antibody structure may be produced, which fragments possess one or more immunoglobulin activities (e.g., complement fixation activity). These polypeptide fragments may be produced by proteolytic cleavage of intact antibodies by methods well known in the art, or by inserting stop codons at the desired locations in vectors using site-

directed mutagenesis, such as after CH1 to produce Fab fragments or after the hinge region to produce $F(ab')_2$ fragments. Single chain antibodies may be produced by joining VL and VH with a DNA linker.

As stated previously, the polynucleotides will be expressed in hosts after the sequences have been operably linked to (i.e., positioned to ensure the functioning of) an expression control sequence. These expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Commonly, expression vectors will contain selection markers, e.g., tetracycline or neomycin, to permit detection of those cells transformed with the desired DNA sequences.

E. coli is a prokaryotic host useful particularly for cloning the polynucleotides of the present invention. Other microbial hosts suitable for use include bacilli, such as Bacillus subtilus, and other enterobacteriaceae, such as Salmonella, Serratia, and various Pseudomonas species. In these prokaryotic hosts, one can also make expression vectors, which will typically contain expression control sequences compatible with the host cell (e.g., an origin of replication). In addition, any of a number of well-known promoters may be present, such as the lactose promoter system, a tryptophan (trp) promoter system, a beta-lactamase promoter system, or a promoter system from phage lambda. The promoters will typically control expression, optionally with an operator sequence, and have ribosome binding site sequences and the like, for initiating and completing transcription and translation.

Other microbes, such as yeast, may also be used for expression. Saccharomyces is a preferred host, with suitable vectors having expression control sequences, such as promoters, including 3-phosphoglycerate kinase or other glycolytic enzymes, and an origin of replication, termination sequences and the like as desired.

In addition to microorganisms, mammalian tissue cell culture may also be used to express and produce the polypeptides of the present invention. Eukaryotic cells are actually preferred, because a number of suitable host cell lines capable of secreting intact immunoglobulins have been developed in the art, and include the CHO cell lines, various COS cell lines, Syrian Hamster Ovary cell lines, HeLa cells, preferably myeloma cell lines, transformed B-cells, human embryonic kidney cell lines, or hybridomas. Expression vectors for these cells can include expression control sequences, such as an

origin of replication, a promoter, an enhancer, and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. Preferred expression control sequences are promoters derived from immunoglobulin genes, SV40, Adenovirus, Bovine Papilloma Virus, cytomegalovirus and the like.

The vectors containing the polynucleotide sequences of interest (e.g., the heavy and light chain encoding sequences and expression control sequences) can be transferred into the host cell by well-known methods, which vary depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment or electroporation may be used for other cellular hosts.

Once expressed, the antibodies can be purified according to standard procedures, including ammonium sulfate precipitation, ion exchange, affinity, reverse phase, hydrophobic interaction column chromatography, gel electrophoresis, and the like. Substantially pure immunoglobulins of at least about 90 to 95% homogeneity are preferred, and 98 to 99% or more homogeneity most preferred, for pharmaceutical uses. Once purified, partially or to homogeneity as desired, the polypeptides may then be used therapeutically or prophylactically, as directed herein.

The antibodies (including immunologically reactive fragments) are administered to a subject at risk for or exhibiting Aβ-related symptoms or pathology such as clinical or pre-clinical Alzheimer's disease, Down's syndrome, or clinical or pre-clinical amyloid angiopathy, using standard administration techniques, preferably peripherally (*i.e.* not by administration into the central nervous system) by intravenous, intraperitoneal, subcutaneous, pulmonary, transdermal, intramuscular, intranasal, buccal, sublingual, or suppository administration. Although the antibodies may be administered directly into the ventricular system, spinal fluid, or brain parenchyma, and techniques for addressing these locations are well known in the art, it is not necessary to utilize these more difficult procedures. The antibodies of the invention are effective when administered by the more simple techniques that rely on the peripheral circulation system. The advantages of the present invention include the ability of the antibody to exert its beneficial effects even though not provided directly to the central nervous system itself. Indeed, it has been

demonstrated that the amount of antibody that crosses the blood-brain barrier is ≤0.1% of plasma levels.

The pharmaceutical compositions for administration are designed to be appropriate for the selected mode of administration, and pharmaceutically acceptable excipients such as, buffers, surfactants, preservatives, solubilizing agents, isotonicity agents, stabilizing agents and the like are used as appropriate. Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton PA, latest edition, incorporated herein by reference, provides a compendium of formulation techniques as are generally known to practitioners.

The concentration of the humanized antibody in formulations may range from as low as about 0.1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, and so forth, in accordance with the particular mode of administration selected. Thus, a pharmaceutical composition for injection could be made up to contain in 1 mL of phosphate buffered saline from 1 to 100 mg of the humanized antibody of the present invention. The formulation could be sterile filtered after making the formulation, or otherwise made microbiologically acceptable. A typical composition for intravenous infusion could have a volume as much as 250 mL of fluid, such as sterile Ringer's solution, and 1-100 mg per mL, or more in antibody concentration. Therapeutic agents of the invention can be frozen or lyophilized for storage and reconstituted in a suitable sterile carrier prior to use. Lyophilization and reconstitution can lead to varying degrees of antibody activity loss (e.g. with conventional immune globulins, IgM antibodies tend to have greater activity loss than IgG antibodies). Dosages may have to be adjusted to compensate. The pH of the formulation will be selected to balance antibody stability (chemical and physical) and comfort to the patient when administered. Generally, pH between 4 and 8 is tolerated.

Although the foregoing methods appear the most convenient and most appropriate for administration of proteins such as humanized antibodies, by suitable adaptation, other techniques for administration, such as transdermal administration and oral administration may be employed provided proper formulation is designed.

In addition, it may be desirable to employ controlled release formulations using biodegradable films and matrices, or osmotic mini-pumps, or delivery systems based on dextran beads, alginate, or collagen.

In summary, formulations are available for administering the antibodies of the invention and are well-known in the art and may be chosen from a variety of options.

Typical dosage levels can be optimized using standard clinical techniques and will be dependent on the mode of administration and the condition of the patient.

The following examples are intended to illustrate but not to limit the invention.

The examples hereinbelow employ, among others, a murine monoclonal antibody designated "10D5" which was originally prepared by immunization with a peptide composed of residues 1-28 of human Aβ peptide. As the examples here describe experiments conducted in murine systems, the use of murine monoclonal antibodies is satisfactory. However, in the treatment methods of the invention intended for human use, humanized forms of the antibodies with the immunospecificity corresponding to that of antibody 10D5 are preferred.

Example 1

Synthesis of Humanized Antibody 10D5

Cells and antibodies. Mouse myeloma cell line Sp2/0 was obtained from ATCC (Manassas, VA) and maintained in DME medium containing 10% FBS (Cat # SH30071.03, HyClone, Logan, UT) in a 37°C CO_2 incubator. Mouse 10D5 hybridoma cells were first grown in RPMI-1640 medium containing 10% FBS (HyClone), 10 mM HEPES, 2 mM glutamine, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate, 25 μg/ml gentamicin, and then expanded in serum-free media (Hybridoma SFM, Cat # 12045-076, Life Technologies, Rockville, MD) containing 2% low Ig FBS (Cat # 30151.03, HyClone) to a 1.5 liter volume in roller bottles. Mouse monoclonal antibody 10D5 (Mu10D5) was purified from the culture supernatant by affinity chromatography using a protein-G Sepharose column. Biotinylated Mu10D5 was prepared using EZ-Link Sulfo-NHS-LC-LC-Biotin (Cat # 21338ZZ, Pierce, Rockford, IL).

Cloning of variable region cDNAs. Total RNA was extracted from approximately 10^7 hybridoma cells using TRIzol reagent (Cat. # 15596-026, Life Technologies) and poly(A)$^+$ RNA was isolated with the PolyATract mRNA Isolation System (Cat. # Z5310, Promega, Madison, WI) according to the suppliers' protocols. Double-stranded cDNA was synthesized using the SMART™RACE cDNA Amplification Kit (Cat. # K1811-1, Clontech, Palo Alto, CA) following the supplier's protocol. The variable region cDNAs

for the light and heavy chains were amplified by polymerase chain reaction (PCR) using 3' primers that anneal respectively to the mouse kappa and gamma chain constant regions, and a 5' universal primer provided in the SMART™RACE cDNA Amplification Kit. For VL PCR, the 3' primer has the sequence:

5'-TATAGAGCTCAAGCTTGGATGGTGGGAAGATGGATACAGTTGGTGC-3'
[SEQ ID NO:13]

with residues 17- 46 hybridizing to the mouse Ck region. For VH PCR, the 3' primers have the degenerate sequences:

```
                                        A       G   T
5'-TATAGAGCTCAAGCTTCCAGTGGATAGACCGATGGGGCTGTCGTTTTGGC-3'
                                        T
```

[SEQ ID NO:14]

with residues 17 - 50 hybridizing to mouse gamma chain CH1. The VL and VH cDNAs were subcloned into pCR4Blunt-TOPO vector (Cat. # 45-0031, Invitrogen, Carlsbad, CA) for sequence determination. DNA sequencing was carried out by PCR cycle sequencing reactions with fluorescent dideoxy chain terminators (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. The sequencing reactions were analyzed on a Model 377 DNA Sequencer (Applied Biosystems).

Construction of humanized 10D5 (Hu10D5) variable regions. Humanization of the mouse antibody V regions was carried out as outlined by Queen et al., 1989, *op. Cit.* The human V region framework used as acceptor for Mu10D5 CDRs was chosen based on sequence homology. The computer programs ABMOD and ENCAD [Levitt, M., J. Mol. Biol. 168:595-620 (1983)] were used to construct a molecular model of the variable regions. Amino acids in the humanized V regions that were predicted to have contact with CDRs were substituted with the corresponding residues of Mu10D5. This was done at residue 98 in the heavy chain and at residues 41 and 51 in the light chain. The amino acids in the humanized V region that were found to be rare in the same V-region subgroup were changed to the consensus amino acids to eliminate potential immunogenicity. This was done at residues 42 and 44 in the light chain and at residue 24 in the heavy chain.

The light and heavy chain variable region genes were constructed and amplified using eight overlapping synthetic oligonucleotides ranging in length from approximately 65 to 80 bases [He, X. Y., et al., J. Immunol. 160: 1029-1035 (1998)]. The oligonucleotides were annealed pairwise and extended with the Klenow fragment of DNA polymerase I, yielding four double-stranded fragments. The resulting fragments were denatured, annealed pairwise, and extended with Klenow, yielding two fragments. These fragments were denatured, annealed pairwise, and extended once again, yielding a full-length gene. The resulting product was amplified by PCR using the Expand High Fidelity PCR System (Cat. # 1 732 650, Roche Molecular Biochemicals, Indianapolis, IN). The PCR-amplified fragments were gel-purified and cloned into pCR4Blunt-TOPO vector. After sequence confirmation, the VL and VH genes were digested with MluI and XbaI, gel-purified, and subcloned respectively into vectors for expression of light and heavy chains to make pVk-Hu10D5 and pVg1-Hu10D5 [Co, M. S., et al., J. Immunol. 148:1149-1154 (1992)]. The mature humanized 10D5 antibody expressed from these plasmids has the light chain of SEQ ID NO:11 and the heavy chain of SEQ ID NO:12.

Stable transfection. Stable transfection into mouse myeloma cell line Sp2/0 was accomplished by electroporation using a Gene Pulser apparatus (BioRad, Hercules, CA) at 360 V and 25 μF as described (Co, et al., 1992, *op. cit.*). Before transfection, pVk-Hu10D5 and pVg1-Hu10D5 plasmid DNAs were linearized using FspI. Approximately 10^7 Sp2/0 cells were transfected with 20 μg of pVk-Hu10D5 and 40 μg of pVg1-Hu10D5. The transfected cells were suspended in DME medium containing 10% FBS and plated

into several 96-well plates. After 48 hr, selection media (DME medium containing 10% FBS, HT media supplement, 0.3 mg/ml xanthine and 1 μg/ml mycophenolic acid) was applied. Approximately 10 days after the initiation of the selection, culture supernatants were assayed for antibody production by ELISA as shown below. High yielding clones were expanded in DME medium containing 10% FBS and further analyzed for antibody expression. Selected clones were then adapted to growth in Hybridoma SFM.

Measurement of antibody expression by ELISA. Wells of a 96-well ELISA plate (Nunc-Immuno plate, Cat # 439454, NalgeNunc, Naperville, IL) were coated with 100 μl of 1 μg/ml goat anti-human IgG, Fc γ fragment specific, polyclonal antibodies (Cat. # 109-005-098, Jackson ImmunoResearch, West Grove, PA) in 0.2 M sodium carbonate-bicarbonate buffer (pH 9.4) overnight at 4°C. After washing with Washing Buffer (PBS containing 0.1% Tween 20), wells were blocked with 400 μl of Superblock Blocking Buffer (Cat # 37535, Pierce) for 30 min and then washed with Washing Buffer. Samples containing Hu10D5 were appropriately diluted in ELISA Buffer (PBS containing 1% BSA and 0.1% Tween 20) and applied to ELISA plates (100 μl per well). As a standard, humanized anti-CD33 IgG1 monoclonal antibody HuM195 (Co, *et al.*, 1992, *op. cit.*) was used. The ELISA plate was incubated for 2 hr at room temperature and the wells were washed with Washing Buffer. Then, 100 μl of 1/1,000-diluted HRP-conjugated goat anti-human kappa polyclonal antibodies (Cat # 1050-05, Southern Biotechnology, Birmingham, AL) in ELISA Buffer was applied to each well. After incubating for 1 hr at room temperature and washing with Washing Buffer, 100 μl of ABTS substrate (Cat #s 507602 and 506502, Kirkegaard and Perry Laboratories, Gaithersburg, MD) was added to each well. Color development was stopped by adding 100 μl of 2% oxalic acid per well. Absorbance was read at 415 nm using an OPTImax microplate reader (Molecular Devices, Menlo Park, CA).

Purification of Hu10D5. One of the high Hu10D5-expressing Sp2/0 stable transfectants (clone #1) was adapted to growth in Hybridoma SFM and expanded to 2 liters in roller bottles. Spent culture supernatant was harvested when cell viability reached 10% or below and loaded onto a protein-A Sepharose column. The column was washed with PBS before the antibody was eluted with 0.1 M glycine-HCl (pH 2.8), 0.1 M NaCl. The eluted protein was dialyzed against 3 changes of 2 liters of PBS and filtered through a 0.2 μm filter prior to storage at 4°C. Antibody concentration was determined

by measuring absorbance at 280 nm (1 mg/ml = 1.4 A_{280}). SDS-PAGE in Tris-glycine buffer was performed according to standard procedures on a 4-20% gradient gel (Cat # EC6025, Novex, San Diego, CA). Purified humanized 10D5 antibody is reduced and run on an SDS- PAGE gel. The whole antibody shows two bands of approximate molecular weights 25 kDa and 50 kDa. These results are consistent with the molecular weights of the light chain and heavy chain, or with the molecular weight of the chain(s) comprising a fragment, calculated from their amino acid compositions.

Example 2

In vitro binding properties of humanized 10D5 antibody

The binding efficacy of humanized 10D5 antibody, synthesized and purified as described above, was compared with the mouse 10D5 antibody using biotinylated mouse 10D5 antibody in a comparative ELISA. Wells of a 96-well ELISA plate (Nunc-Immuno plate, Cat # 439454, NalgeNunc) were coated with 100 µl of β-amyloid peptide (1-42) in 0.2 M sodium carbonate/bicarbonate buffer (pH 9.4) (1 µg/mL) overnight at 4°C.

After washing the wells with phosphate buffered saline (PBS) containing 0.1% Tween 20 (Washing Buffer) using an ELISA plate washer, the wells were blocked by adding 300 µL of SuperBlock reagent (Pierce) per well. After 30 minutes of blocking, the wells were washed with Washing Buffer and excess liquid was removed.

A mixture of biotinylated Mu10D5 (0.4 µg/ml final concentration) and competitor antibody (Mu10D5 or Hu10D5; starting at 1000 µg/ml final concentration and serial 3-fold dilutions) in ELISA Buffer were added in triplicate in a final volume of 100 µl per well. As a no-competitor control, 100 µl of 0.4 µg/ml biotinylated Mu10D5 was added. As a background control, 100 µl of ELISA Buffer was added. The ELISA plate was incubated at room temperature for 90 min. After washing the wells with Washing Buffer, 100 µl of 10 µg/ml HRP-conjugated streptavidin (Cat # 21124, Pierce) was added to each well. The plate was incubated at room temperature for 30 min and washed with Washing Buffer. For color development, 100 µl/well of ABTS Peroxidase Substrate (Kirkegaard & Perry Laboratories) was added. Color development was stopped by adding 100 µl/well of 2% oxalic acid. Absorbance was read at 415 nm. The absorbances were plotted against the log of the competitor concentration, curves were fit to the data points (using Prism) and the IC50 was determined for each antibody using methods well-known in the art.

The mean IC50 for mouse 10D5 was 23.4 μg/mL (three separate experiments, standard deviation =5.5 μg/mL) and for humanized 10D5 was 49.1 μg/mL (three separate experiments, standard deviation = 11.8 μg/mL). A second set of three experiments was carried out, essentially as described above, and the mean IC50 for mouse 10D5 was determined to be 20 μg/mL (SD = 1 μg/mL) and for humanized 10D5, the IC50 was determined to be 16 μg/mL (SD = 0.6 μg/mL). On the basis of these results, we conclude that humanized 10D5 has binding properties that are very similar to those of the mouse antibody 10D5. Therefore, we expect that humanized 10D5 has very similar *in vitro* and *in vivo* activities compared with mouse 10D5 and will exhibit in humans the same effects demonstrated with mouse 10D5 in mice.

Example 3

In vitro binding properties of mouse and humanized antibodies 10D5

Antibody affinity (KD = Kd / Ka) was determined using a BIAcore biosensor 2000 and data analyzed with BIAevaluation (v. 3.1) software. A capture antibody (rabbit anti-mouse Ig or anti-human Ig) was coupled via free amine groups to carboxyl groups on flow cell 2 of a biosensor chip (CM5) using N-ethyl-N-dimethylaminopropyl carbodiimide and N-hydroxysuccinimide (EDC/NHS). A non-specific rabbit IgG was coupled to flow cell 1 as a background control. Monoclonal antibodies were captured to yield 300 resonance units (RU). Amyloid-beta 1-40 or 1-42 (Biosource International, Inc.) was then flowed over the chip at decreasing concentrations (1000 to 0.1 times KD). To regenerate the chip, bound anti-Aβ antibody was eluted from the chip using a wash with glycine-HCl (pH 2). A control injection containing no amyloid-beta served as a control for baseline subtraction. Sensorgrams demonstrating association and dissociation phases were analyzed to determine Kd and Ka. The affinity (KD) of mouse antibody 10D5 for Aβ 1-40 was determined to be 390 nM, and the affinity of humanized 10D5, prepared essentially as described in Example 1, was determined to be 209 nM. Affinity for Aβ 1-42 was biphasic for both mouse 10D5 and humanized 10D5. For mouse 10D5, the affinities for Aβ 1-42 were 0.57 nM and 4950 nM. Humanized 10D5 had affinities for Aβ 1-42 of 0.19 nM and 1020 nM.

Example 4

Epitope mapping of mouse and humanized 10D5

The BIAcore is an automated biosensor system for measuring molecular interactions [Karlsson R., *et al. J. Immunol. Methods* 145:229-240 (1991)]. The advantage of the BIAcore over other binding assays is that binding of the antigen can be measured without having to label or immobilize the antigen (i.e. the antigen maintains a more native conformation). The BIAcore methodology was used to assess the binding of various amyloid-beta peptide fragments to either mouse 10D5 or humanized 10D5 (prepared substantially as described in Example 1). All dilutions were made with HEPES buffered saline containing Tween 20. A single concentration of a variety of fragments of human Aβ or mouse Aβ 1-40 (BioSource International) was used. Human amyloid beta fragments 1-10 and 1-20 bound to mouse 10D5 and to humanized 10D5, while human Aβ fragments 10-20 and 16-25 did not bind to either antibody. Neither mouse 10D5 nor humanized 10D5 bound mouse Aβ 1-40. Using this methodology, the binding epitope for both mouse and humanized 10D5 appears to be between amino acids 1 and 10 of human Aβ.

Example 5

In vivo experiments with 10D5

Unless otherwise stated, all studies used PDAPP mice, and all injections were intraperitoneal (i.p.) In general, a control group of mice received injections of saline. In some cases, another control group received injections of a non-specific mouse IgG preparation.

Six weeks of weekly injection of 360 μg of 10D5 in old mice (24 month) raised soluble $A\beta_{total}$ in hippocampus by 16% and Aβ 1-42 in hippocampus by 21%, while lowering hippocampal insoluble $A\beta_{total}$ by 24% and Aβ 1-42 by 26% (no statistically significant difference; 9 animals per control group and 10 animals per antibody group). In the cortex, mean insoluble $A\beta_{total}$ was lower by 27% and Aβ 1-42 by 29%, while mean insoluble Aβ 1-40 increased by 7% (no statistically significant differences).

In hemizygous, 4 month old mice, administration of 360 μg 10D5 per animal: 1) raised average plasma Aβ 1-40 and Aβ 1-42 levels approximately 3-fold by 24 hours after administration; and 2) had no significant effect on soluble Aβ 1-40 in the cortex after 24

hours compared with saline control (no differences were statistically significant; 5 animals per group).

Administration of 360 μg of 10D5 per animal (5 animals per group, saline control): 1) raised average plasma Aβ 1-40 and Aβ 1-42 levels approximately 14-fold and 19-fold, respectively by 24 hours after administration; 2) had no consistent or significant effect on soluble or insoluble Aβ 1-40, Aβ 1-42, or $A\beta_{total}$ in the cortex or hippocampus after 24 hours; 3) lowered soluble Aβ 1-40, Aβ 1-42, and $A\beta_{total}$ in the cerebellum by 50% ($p<0.05$), 33%, and 13%, respectively; and 4) lowered insoluble Aβ 1-40, Aβ 1-42, and $A\beta_{total}$ in the cerebellum by 53% ($p<0.001$), 46% ($p<0.001$), and 30% ($p<0.01$), respectively.

In young mice, administration of 360 μg of 10D5 per animal (5 per group): 1) raised average plasma Aβ 1-42 levels approximately 33% by 24 hours after administration; and 2) in the cortex, raised soluble Aβ 1-40 3.4-fold ($p<0.001$), lowered soluble Aβ 1-42 by 22% ($p<0.05$), lowered insoluble Aβ 1-40 about 10% and increased insoluble Aβ 1-42 about 12%.

We claim:

1. Humanized 10D5 antibody.

2. A humanized antibody, or fragment thereof, comprising a humanized light chain comprising three light chain complementarity determining regions (CDRs) from the mouse monoclonal antibody 10D5 and a light chain variable region framework sequence from a human immunoglobulin light chain; and a humanized heavy chain comprising three heavy chain CDRs from the mouse monoclonal antibody 10D5 and a heavy chain variable region framework sequence from a human immunoglobulin heavy chain; wherein the light chain CDRs have the following amino acid sequences:

light chain CDR1:

```
1               5                  10                  15
Arg Ser Ser Gln Asn Ile Ile His Ser Asn Gly Asn Thr Tyr Leu Glu
(SEQ ID NO:1)
```

light chain CDR2:

```
1               5
Lys Val Ser Asn Arg Phe Ser  (SEQ ID NO:2)
```

light chain CDR3:

```
1               5
Phe Gln Gly Ser His Val Pro Leu Thr  (SEQ ID NO:3)
```

and the heavy chain CDRs have the following amino acid sequences:

heavy chain CDR1:

```
1               5
Thr Ser Gly Met Gly Val Ser (SEQ ID NO:4)
```

heavy chain CDR2:

```
1               5                  10                  15
His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ser Leu Lys Ser
(SEQ ID NO:5)
```

and, heavy chain CDR3:

```
1               5                  10
Arg Pro Ile Thr Pro Val Leu Val Asp Ala Met Asp Tyr  (SEQ ID NO:6).
```

3. A humanized antibody or fragment thereof comprising a humanized light chain variable region having the sequence of SEQ ID NO:7 and a humanized heavy variable region having the sequence of SEQ ID NO:8.

4. The humanized antibody or fragment thereof of claim 3 having a light chain variable region of the sequence given by SEQ ID NO:9 and a heavy chain variable region given by SEQ ID NO:10.

5. The humanized antibody or fragment thereof of claim 3 having a light chain of the sequence given by SEQ ID NO:11 and a heavy chain of the sequence given by SEQ ID NO:12.

6. An antibody fragment obtainable by enzymatic cleavage of the humanized antibody of any one of claims 1 - 5.

7. An Fab or $F(ab')_2$ fragment of any one of the humanized antibodies of claims 1 - 5.

8. The $F(ab')_2$ fragment of claim 7.

9. The Fab fragment of claim 7.

10. The humanized antibody or fragment of any one of claims 1 – 9, which is a single chain antibody.

11. The humanized antibody or fragment of any one of claims 1 - 10 that is an IgG_1 immunoglobulin isotype.

12. The humanized antibody or fragment of any one of claims 1 - 11, wherein the antibody or fragment thereof is produced in a host cell selected from the group consisting of a myeloma cell, a chinese hamster ovary cell, a syrian hamster ovary cell, and a human embryonic kidney cell.

13. A polynucleotide compound, comprising a sequence coding for the light chain or the heavy chain of the humanized antibody of any one of claims 1 - 12, or a fragment thereof.

14. A polynucleotide sequence, which when expressed in a suitable host cell, yields an antibody of any one of claims 1 – 12.

15. The polynucleotide of claim 13 or 14 selected from the group consisting of SEQ ID NO: 15, SEQ ID NO: 17, and a polynucleotide comprising a sequence that codes for the light chain variable region given by SEQ ID NO:7, SEQ ID NO:9, or SEQ ID NO: 11.

16. The polynucleotide of claim 13 or 14 selected from the group consisting of SEQ ID NO:16, SEQ ID NO:18, and a polynucleotide comprising a sequence that codes for the heavy chain variable region given by SEQ ID NO:8, SEQ ID NO:10, or SEQ ID NO:12.

17. An expression vector for expressing the antibody of any one of claims 1 - 12 comprising the polynucleotide sequence of any one of claims 13 - 16.

18. A cell transfected with the expression vector of claim 17.

19. A cell transfected with two expression vectors of claim 17, wherein a first vector comprises the polynucleotide sequence coding for the light chain and a second vector comprises the sequence coding for the heavy chain.

20. A cell that is capable of expressing the humanized antibody or fragment of any one of claims 1 – 12.

21. The cell of any one of claims 18 – 20, wherein the cell is selected from the group consisting of a myeloma cell, a chinese hamster ovary cell, a syrian hamster ovary cell, and a human embryonic kidney cell.

22. A pharmaceutical composition comprising the humanized antibody or fragment of any one of claims 1 – 12, and a pharmaceutically acceptable excipient.

23. A method of treating Down s syndrome, clinical or pre-clinical Alzheimer's disease, or clinical or pre-clinical cerebral amyloid angiopathy in a human subject, comprising administering to the human subject an effective amount of a humanized antibody or fragment of any one of claims 1 – 12.

24. A method to inhibit the formation of Aβ plaque in the brain of a human subject, comprising administering to the human subject an effective amount of the humanized antibody or fragment of any one of claims 1 – 12.

25. A method to reduce Aβ plaque in the brain of a human subject, comprising administering to the human subject an effective amount of a humanized antibody or fragment of any one of claims 1 – 12.

26. The method of either of claims 24 – 25, wherein the subject is diagnosed with clinical or pre-clinical Alzheimer's disease, Down's syndrome, or clinical or pre-clinical cerebral amyloid angiopathy.

27. The method of any one of claims 24 – 25, wherein the subject is not diagnosed with clinical or pre-clinical Alzheimer's disease, Down's syndrome, or clinical or pre-clinical cerebral amyloid angiopathy.

28. Use of the humanized antibody or a fragment thereof according to any one of Claims 1 – 12 for the manufacture of a medicament, including prolonged expression of recombinant sequences of the antibody or antibody fragment in human tissues, for treating clinical or pre-clinical Alzheimer's disease, Down's syndrome, or clinical or pre-clinical cerebral amyloid angiopathy.

29. Use of the humanized antibody or fragment of any one of claims 1 – 12 for the manufacture of a medicament for treating Alzheimer's disease.

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Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Xaa Xaa Gln Val
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Val Leu Xaa Xaa Thr Xaa Xaa Asp Pro Val Asp Thr Ala Thr Tyr Tyr
85 90 95

Cys Val Arg Arg Pro Ile Thr Pro Val Leu Val Asp Ala Met Asp Tyr
100 105 110

Trp Gly Gln Gly Thr Xaa Val Thr Val Ser Ser
115 120

<210> 9

<211> 113

<212> PRT

<213> humanized antibody

<400> 9

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile Ile His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Arg Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95

Ser His Val Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg

<210> 10

<211> 123

<212> PRT

<213> humanized antibody

<400> 10

Gln Val Thr Leu Lys Glu Ser Gly Pro Val Leu Val Lys Pro Thr Glu
1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ser
20 25 30

Gly Met Gly Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
35 40 45

Trp Leu Ala His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ser
50 55 60

Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Ser Gln Val
65 70 75 80

Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
85 90 95

Cys Val Arg Arg Pro Ile Thr Pro Val Leu Val Asp Ala Met Asp Tyr
100 105 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 11

<211> 219

<212> PRT

<213> humanized antibody

<400> 11

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile Ile His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Arg Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95

Ser His Val Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
115 120 125

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
130 135 140

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
145 150 155 160

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
165 170 175

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
180 185 190

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
195 200 205

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 12

<211> 453

<212> PRT

<213> humanized antibody

<400> 12

Gln Val Thr Leu Lys Glu Ser Gly Pro Val Leu Val Lys Pro Thr Glu
1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ser
20 25 30

Gly Met Gly Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
35 40 45

Trp Leu Ala His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ser
50 55 60

Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Ser Gln Val
65 70 75 80

Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
85 90 95

Cys Val Arg Arg Pro Ile Thr Pro Val Leu Val Asp Ala Met Asp Tyr
100 105 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
115 120 125

Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
130 135 140

Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
145 150 155 160

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
165 170 175

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
180 185 190

Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
195 200 205

Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
210 215 220

Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
225 230 235 240

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
245 250 255

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
260 265 270

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
275 280 285

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
290 295 300

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
305 310 315 320

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
325 330 335

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
340 345 350

Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
355 360 365

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
370 375 380

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
385 390 395 400

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
405 410 415

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
420 425 430

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
435 440 445

Leu Ser Pro Gly Lys
450

<210> 13

<211> 46

<212> DNA

<213> DNA primer

<400> 13
tatagagctc aagcttggat ggtgggaaga tggatacagt tggtgc 46

<210> 14

<211> 50

<212> DNA

<213> DNA primer

<400> 14
tatagagctc aagcttccag tggatagach gatggggstg tygttttggc 50

<210> 15

<211> 1953

<212> DNA

<213> humanized antibody

<400> 15
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cagcctccat ctcttgcaga tctagtcaga acattataca tagtaatgga aacacctatt 180
tagaatggta cctgcagaaa ccaggccagt ctccaaggct cctgatctac aaagtttcca 240
accgattttc tggggtccca gacaggttca gtggcagtgg atcagggaca gatttcacac 300
tcaagatcag cagagtggag gctgaggatg tgggagttta ttactgcttt caaggttcac 360
atgttccgct cactttcggc ggagggacca aggtggaaat aaaacgtaag tgcactttcc 420
taatctagaa attctaaact ctgagggggt cggatgacgt ggccattctt tgcctaaagc 480
attgagttta ctgcaaggtc agaaaagcat gcaaagccct cagaatggct gcaaagagct 540
ccaacaaaac aatttagaac tttattaagg aatagggga agctaggaag aaactcaaaa 600
catcaagatt ttaaatacgc ttcttggtct ccttgctata attatctggg ataagcatgc 660
tgttttctgt ctgtccctaa catgccctgt gattatccgc aaacaacaca cccaagggca 720
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cttagactta atggaatgtc atgccttatt tacattttta aacaggtact gagggactcc 1800
tgtctgccaa gggccgtatt gagtactttc cacaacctaa tttaatccac actatactgt 1860
gagattaaaa acattcatta aaatgttgca aaggttctat aaagctgaga gacaaatata 1920
ttctataact cagcaatccc acttctagga tcc 1953

<210> 16
<211> 3256
<212> DNA
<213> humanized antibody

<400> 16
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cttccctgca ggatcc 3256

<210> 17
<211> 714
<212> DNA
<213> humanized antibody

<400> 17
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tcttgcagat ctagtcagaa cattatacat agtaatggaa acacctattt agaatggtac 180
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agcaccctga cgctgagcaa agcagactac gagaaacaca aagtctacgc ctgcgaagtc 660
acccatcagg gcctgagctc gcccgtcaca aagagcttca acaggggaga gtgt 714

<210> 18
<211> 1416
<212> DNA
<213> humanized antibody

<400> 18
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tacaacagca cgtaccgtgt ggtcagcgtc ctcaccgtcc tgcaccagga ctggctgaat 1020
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<210> 19
<211> 238
<212> PRT
<213> humanized antibody

<400> 19

Met Lys Leu Pro Val Arg Leu Leu Val Leu Met Phe Trp Ile Pro Ala
1 5 10 15

Ser Ser Ser Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val
20 25 30

Thr Leu Gly Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile
35 40 45

Ile His Ser Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro
50 55 60

Gly Gln Ser Pro Arg Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser
65 70 75 80

Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
85 90 95

Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys
100 105 110

Phe Gln Gly Ser His Val Pro Leu Thr Phe Gly Gly Gly Thr Lys Val
115 120 125

Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro
130 135 140

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu
145 150 155 160

Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn
165 170 175

Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser
180 185 190

Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala
195 200 205

Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly
210 215 220

Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235

<210> 20

<211> 472

<212> PRT

<213> humanized antibody

<400> 20

Met Asp Arg Leu Thr Ser Ser Phe Leu Leu Leu Ile Val Pro Ala Tyr
1 5 10 15

Val Leu Ser Gln Val Thr Leu Lys Glu Ser Gly Pro Val Leu Val Lys
20 25 30

Pro Thr Glu Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu
35 40 45

Ser Thr Ser Gly Met Gly Val Ser Trp Ile Arg Gln Pro Pro Gly Lys
50 55 60

Ala Leu Glu Trp Leu Ala His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr
65 70 75 80

Asn Pro Ser Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys
85 90 95

Ser Gln Val Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala
100 105 110

Thr Tyr Tyr Cys Val Arg Arg Pro Ile Thr Pro Val Leu Val Asp Ala
115 120 125

Met Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser
130 135 140

Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr
145 150 155 160

Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro
165 170 175

Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val
180 185 190

His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser
195 200 205

Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile
210 215 220

Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val
225 230 235 240

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
245 250 255

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
260 265 270

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
275 280 285

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
290 295 300

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
305 310 315 320

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
325 330 335

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
340 345 350

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
355 360 365

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
370 375 380

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
385 390 395 400

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
405 410 415

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
420 425 430

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
435 440 445

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
450 455 460

Ser Leu Ser Leu Ser Pro Gly Lys
465 470